

# Analysis of biofilms on medical explants

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# Challenges of Detecting Biofilms on Medical Implants

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Very difficult if not impossible at all to detect biofilms *in vivo*

- Destructive to patients
- No specific biomarkers
- Different materials, different shapes, different sites
- No approved clinical protocols

Most biofilm analysis on medical devices using models, explants, or discarded devices

Vertes, Hitchins, and Phillips 2012 Analytical Chem

Hall-Stoodley et al. 2012. FEMS Immunol Med Microbiol

# Steps of explants collection

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Device explantation which involves:

- Disinfection of surgical sites with alcohol and/or iodine (DuraPrep, ChloroPrep, PVP-I..)
  - Intravenous or oral delivery of perioperative antimicrobial prophylaxis (3<sup>rd</sup> Gen. Cephalosporin)
  - Irrigation of surgical sites with Ringer's solution containing antibiotics.
  - Device removal and storage under aseptic conditions
- These procedures can alter outcome of detection.

# Methods of analysis of biofilms

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- Culture based methods
- DNA based methods (PCR, DNA sequencing..)
- Imaging methods
  - Confocal Laser Scanning Microscopy with molecular probes (FISH or labeled antibodies)
  - Electron microscopy (TEM, SEM)
- Others

# Culture-based detection methods

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## **Pros:**

- Standard practices
- Several selective media developed
- Direct viable counts

## **Cons:**

- Low sensitivity
- Viable But Non-Culturable (VBNC) state
- Few bacterial species recovered
- No biofilm structure, function

## **Key considerations for culture-based methods:**

**Growth requirements ( $O_2$ , nutrients, cofactors, time)**

**Improve sensitivity (disperse biofilms and revive of cells)**

# Vortexing-Sonication



Superficial swabs generally yield poor results because biofilm bacteria are embedded in EPS, within a tissue and are not easily picked up.

Vortexing-sonication release bacteria from biofilms and improve detection. This method involves:

- Pre-incubation of device in sterile media (37°C, 24 - 48h)
- Removal of spent media
- Vortexing in Ringer's solution for 30s
- Sonication at > 20 KHz for 5 min followed by vortexing
- Centrifugation of sonication fluid at ~ 3000g (RCF) 5-20 min
- Culture of sedimented materials on agar plates

Nelson et al, 2005; Trampuz et al, 2007

# Bacteria detected with foreign body materials



## Infections associated with foreign body material

Contact lens	<i>P. aeruginosa</i> , Gram-positive cocci
Sutures	Staphylococci
Ventilation-associated pneumonia	Gram-negative rods
Mechanical heart valves	Staphylococci
Vascular grafts	Gram-positive cocci
Arteriovenous shunts	Staphylococci
Endovascular catheter infections	Staphylococci
Cerebral spinal fluid-shunts	Staphylococci
Peritoneal dialysis (CAPD) peritonitis	Various species
Urinary catheter infections	<i>E. coli</i> , Gram-negative rods
IUDs	<i>Actinomyces israelii</i> and others
Penile prostheses	Staphylococci
Orthopedic prosthesis	Staphylococci

<sup>a</sup>Adapted, with permission, from Ref. [1].

Fux et al. 2006 Survival strategies of infectious biofilms. Trends in Microbiology  
Otto, 2008 Staphylococcal biofilms. Curr. Top Microbiol. Immunol.

# DNA Based Methods

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DNA amplification-based methods are sensitive and help overcome certain issues in culture based methods.

Methods include:

- PCR amplification and quantitative PCR

- DGGE (Denaturing Gradient Gel Electrophoresis)

- T-RFLP (Terminal restriction fragment length polymorphism)

- SSCP (Single Strand Conformation Polymorphism)

- DNA sequencing



# DNA Based Methods

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## **Pros:**

Sensitive  
Detect more complex  
microbial communities

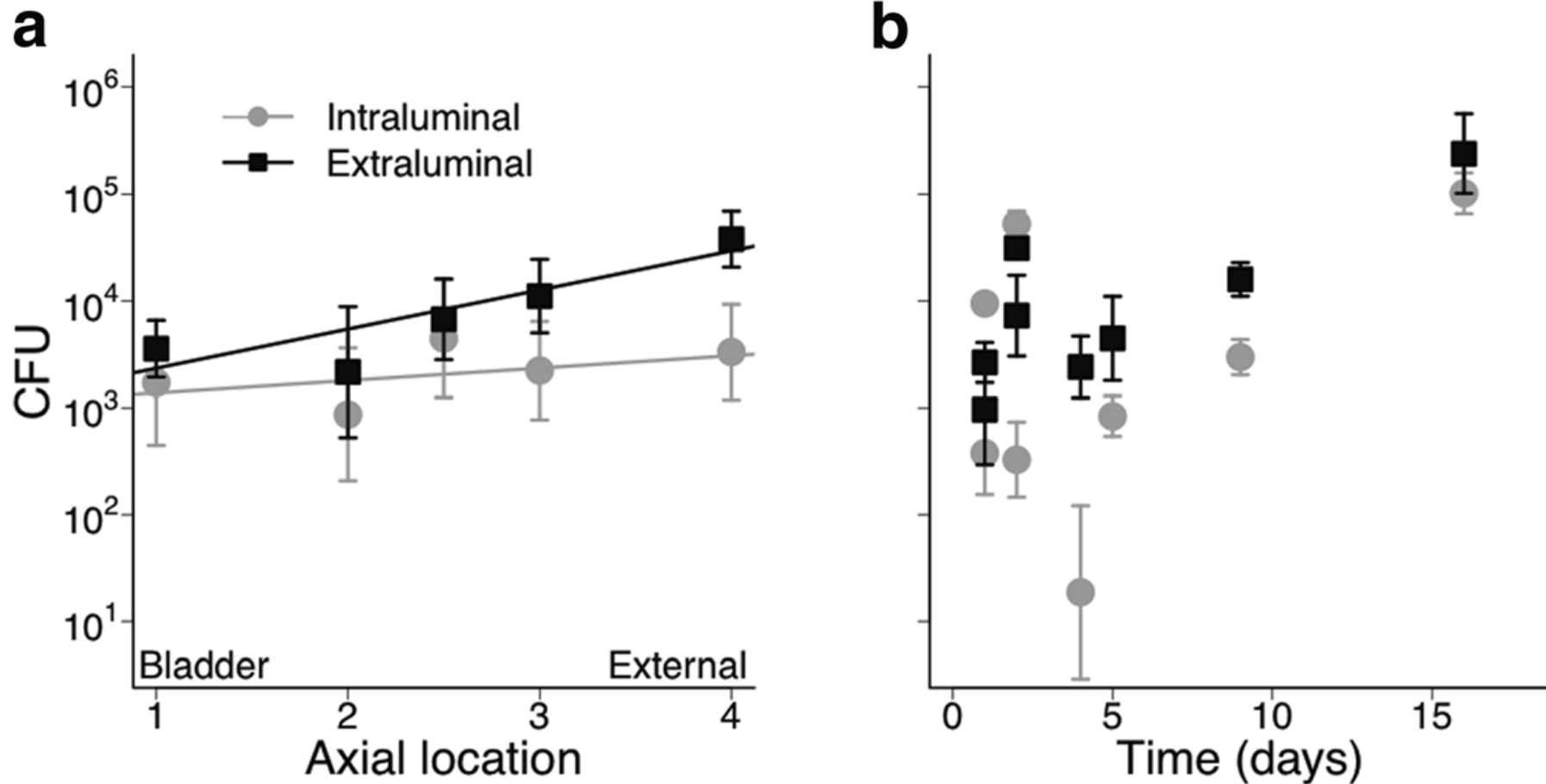
## **Cons:**

Contamination  
False positive  
No differentiation of live/dead cells  
Potential inhibitors

**Appropriate controls to avoid false positives**

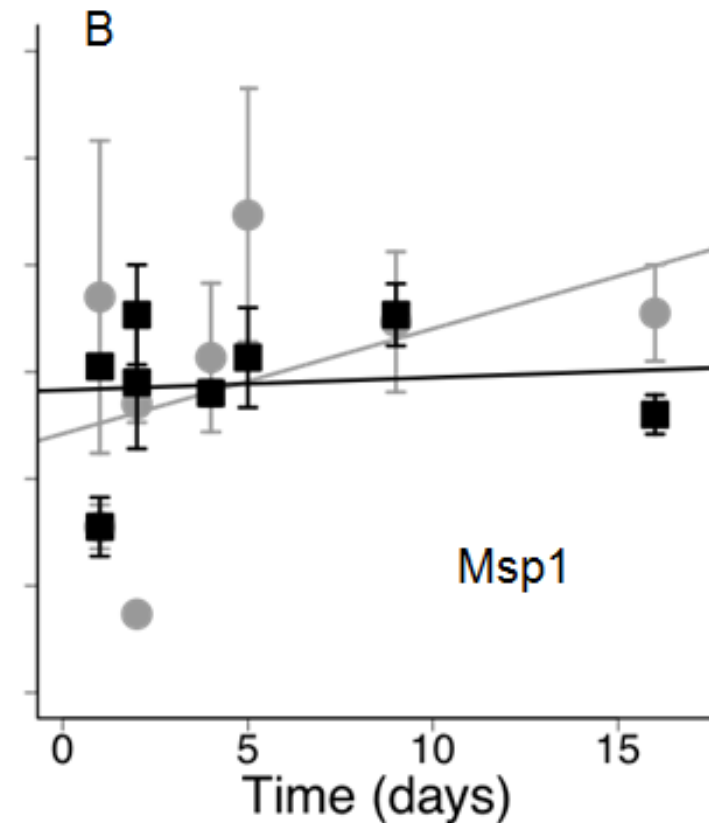
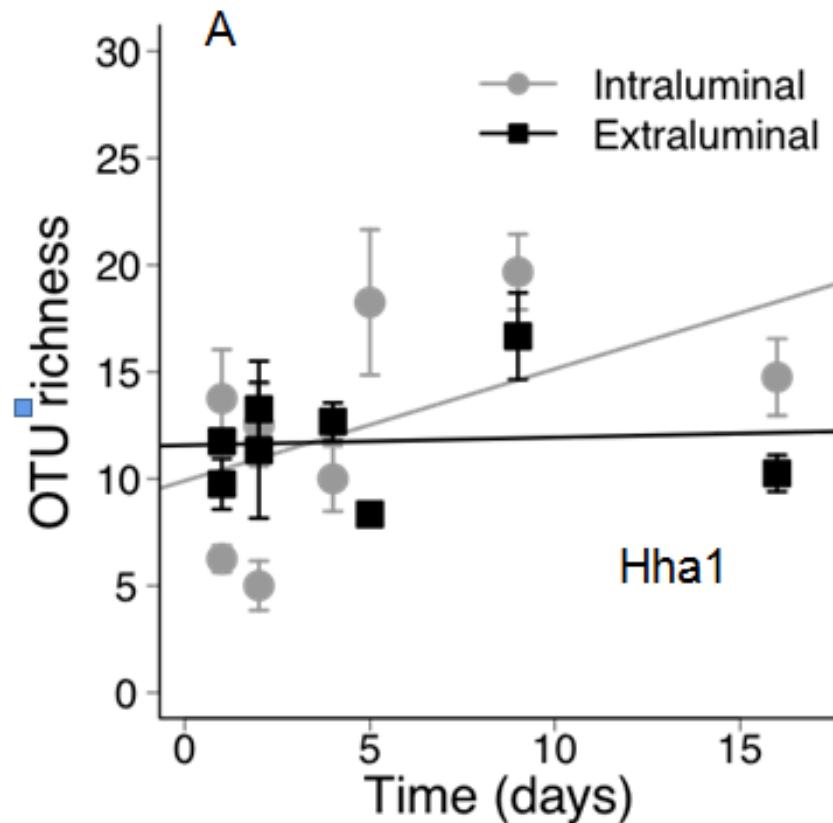
Donlan 2002; Lasen et al 2008

# Biofilm development on urinary catheters



Foxman et al. 2012

# Biofilm development on urinary catheters



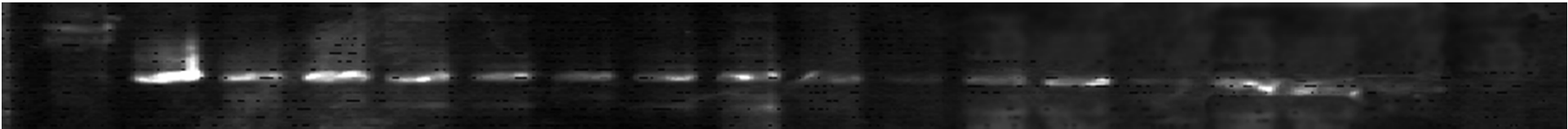
Foxman et al. 2012

# *Biofilms on Hip Joint prosthesis*

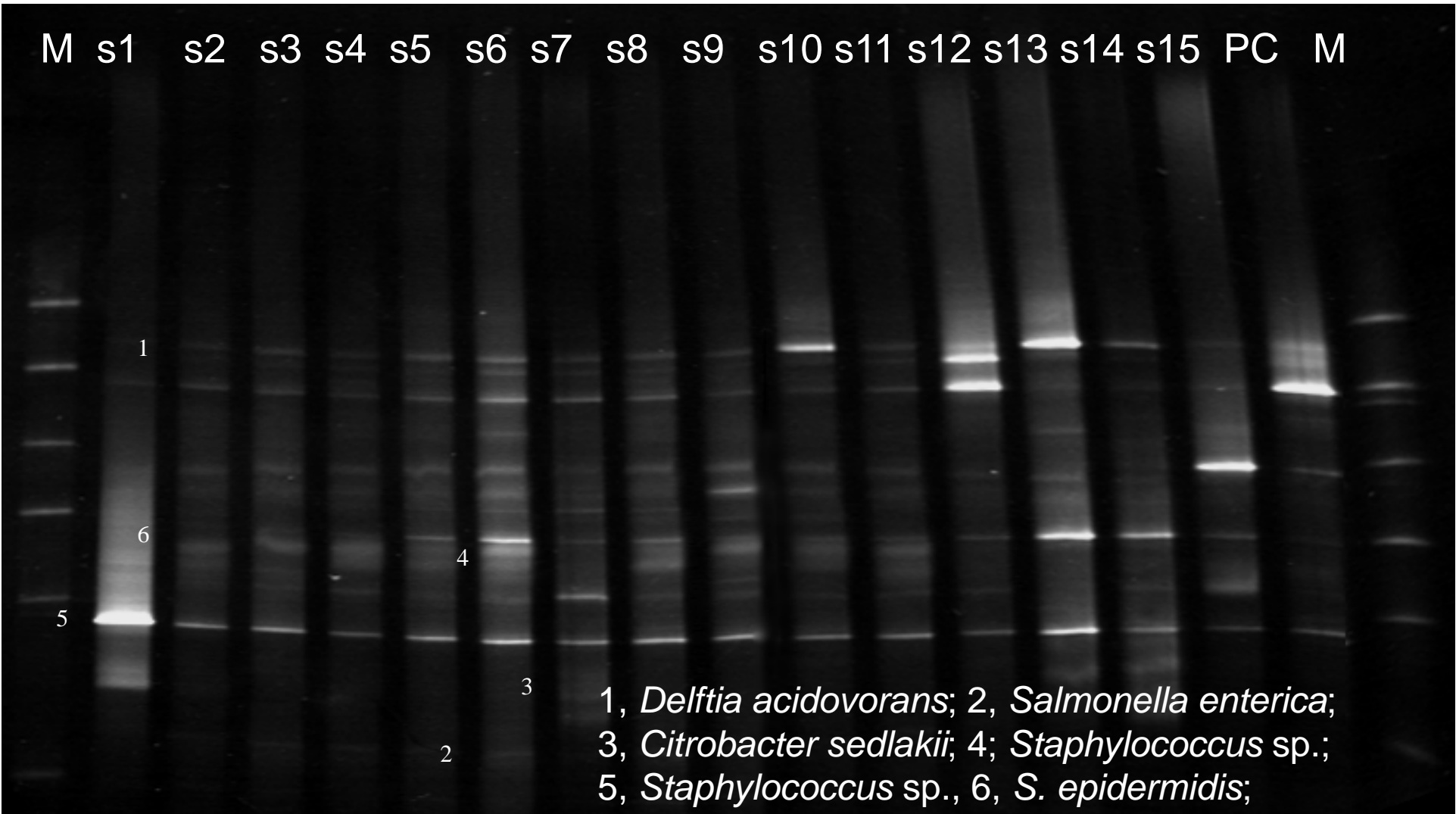


Xi et al, unpublished data

Agarose gel

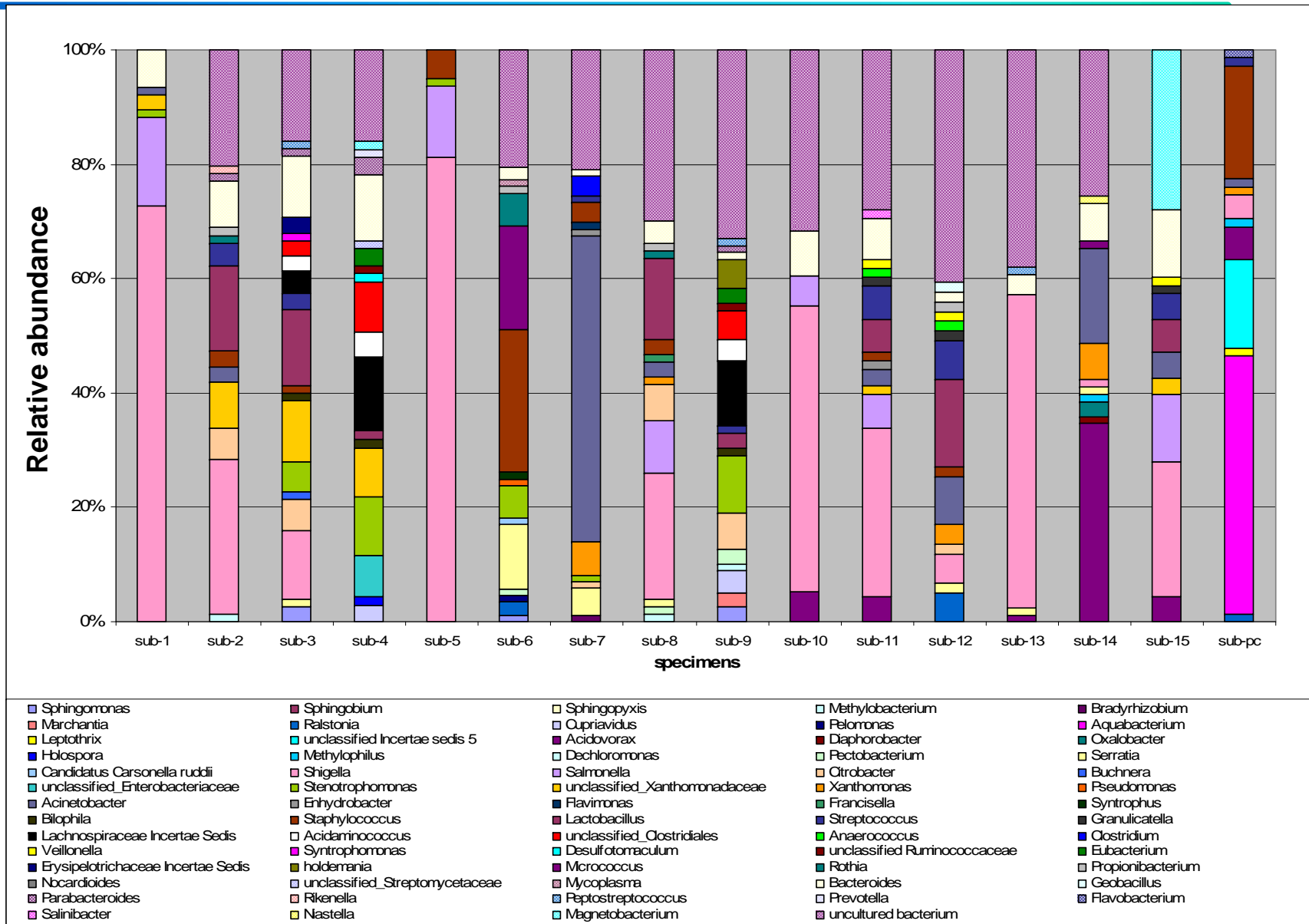


DGGE gel





# Bacterial Community Composition on hip joint prosthesis



**Biofilms developed very quickly  
on medical devices right after  
the use in human body**

**and composed of a community  
of microbial populations**

# Imaging methods with probes/dyes

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Molecular probes labeled with a fluorescent tag can be used to detect biofilm bacteria in infected medical devices.

Dyes staining biofilm matrix, i.e. polysaccharide, protein, DNA.

FISH (Fluorescence In Situ Hybridization) is commonly used:

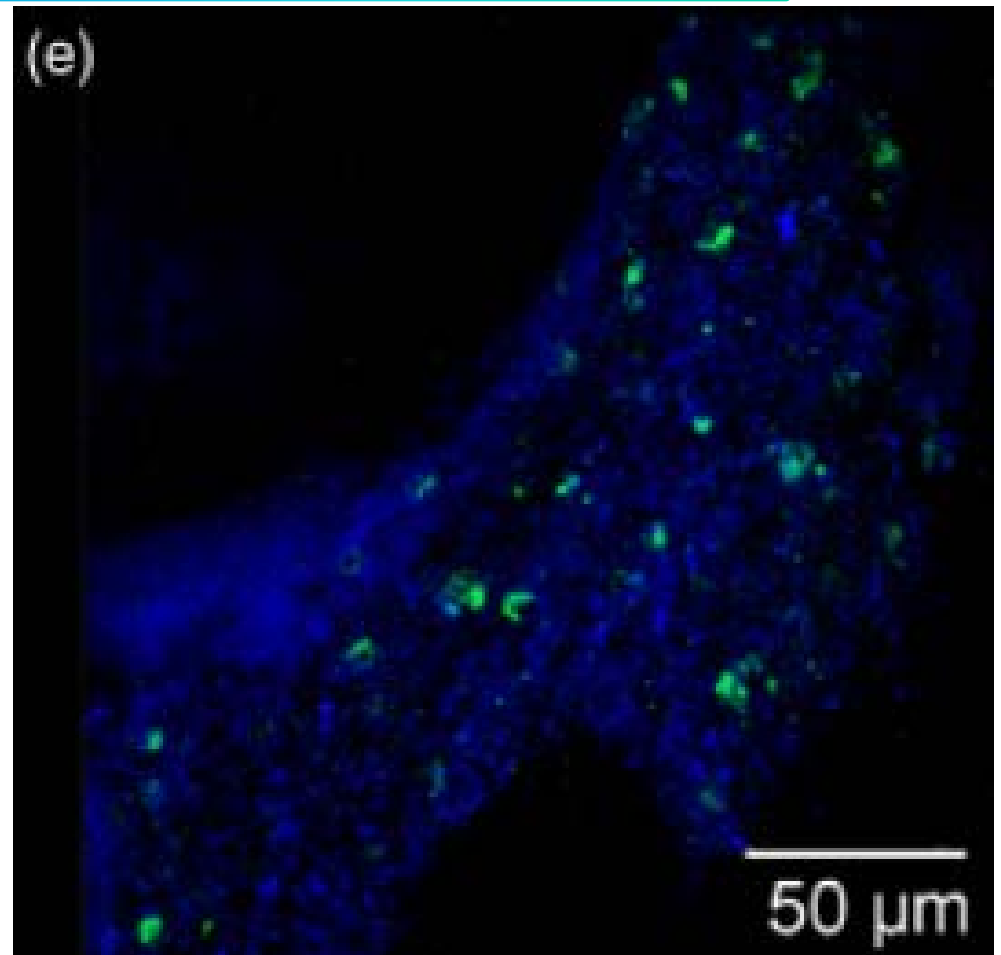
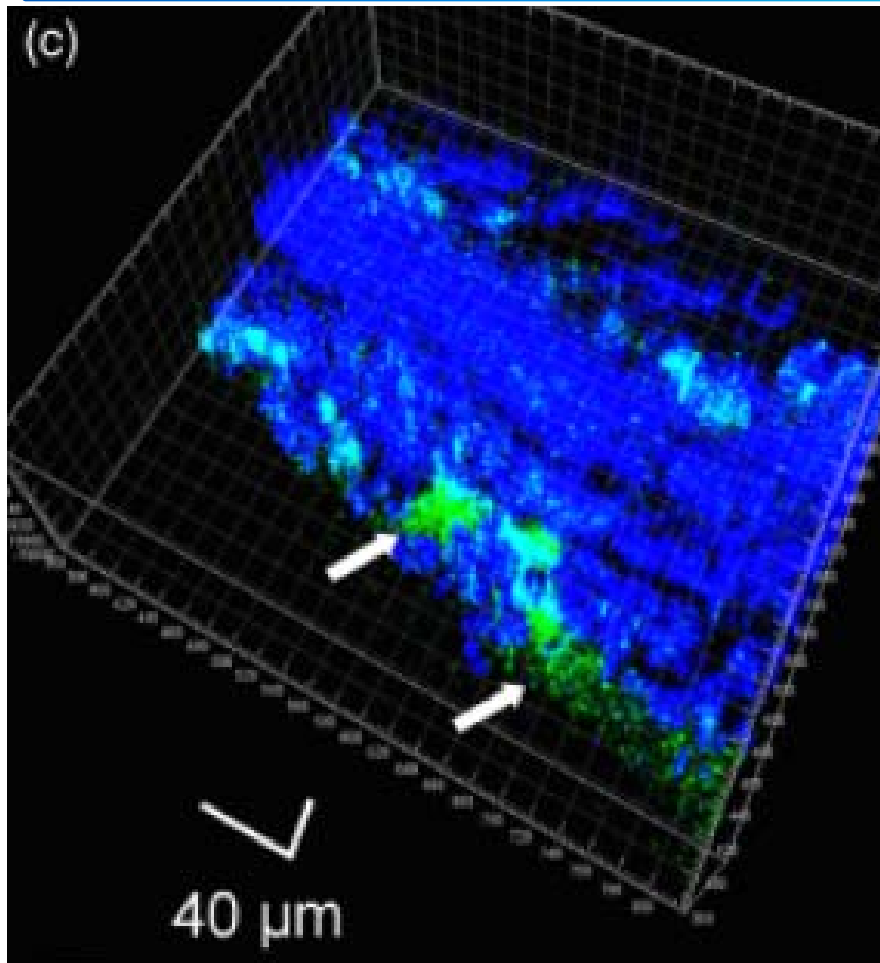
Single stranded 16S rDNA fragment labeled with a fluorescent dye recognizing specific species of biofilm bacteria.

Detection requires high resolution microscopy such as Laser Scanning Confocal Microscope

Difficult to do on explants: different shapes, short imaging depth

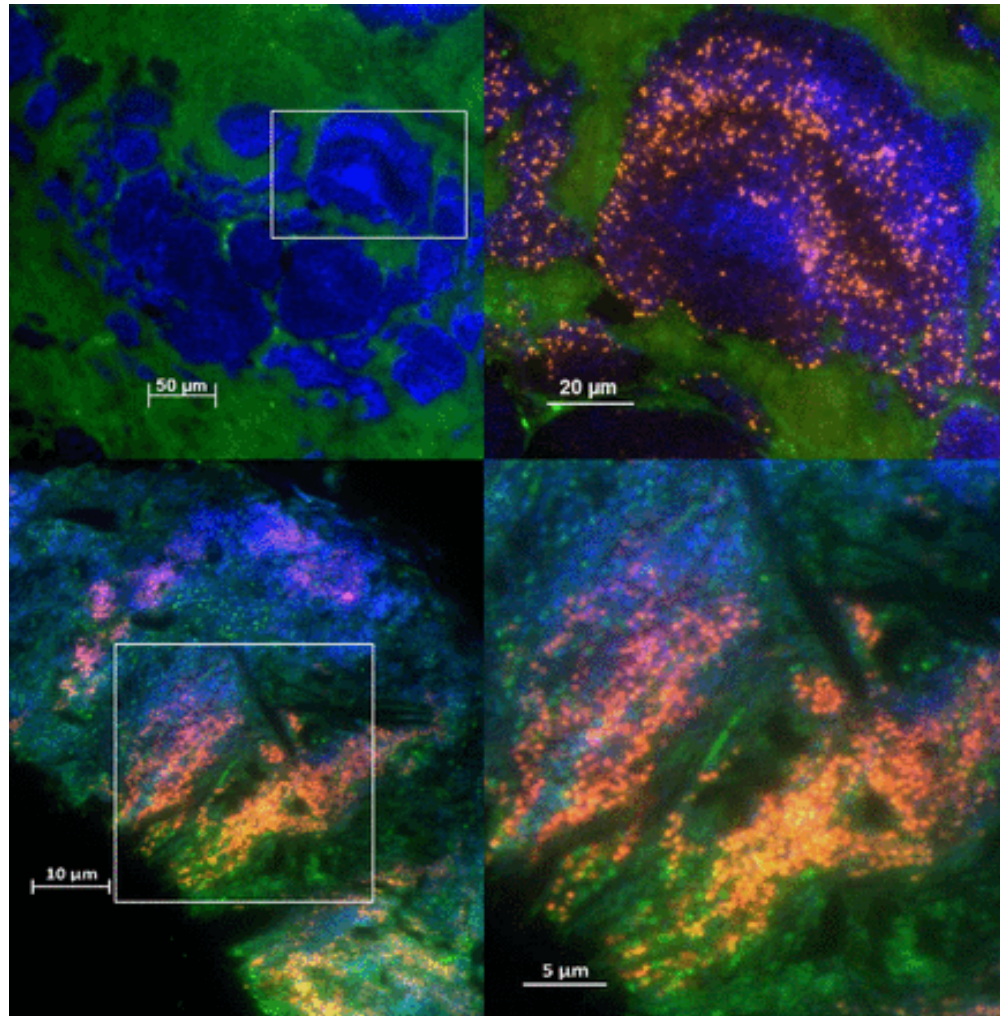


# Biofilms developed on an ankle arthroplasty



Colonization of an ankle arthroplasty (blue) by *Staphylococcus aureus* biofilms (green)  
Stoodley, P. et al. (2011) FEMS Immunology & Medical Microbiology 62(1) 66-74

# FISH and confocal imaging of biofilms on heart valve section



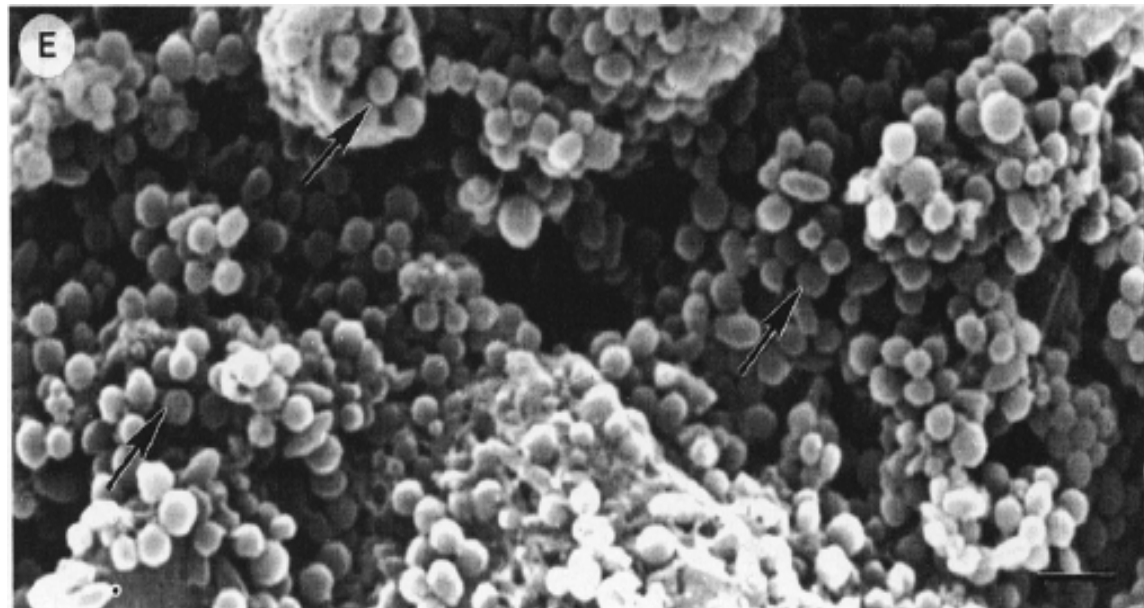
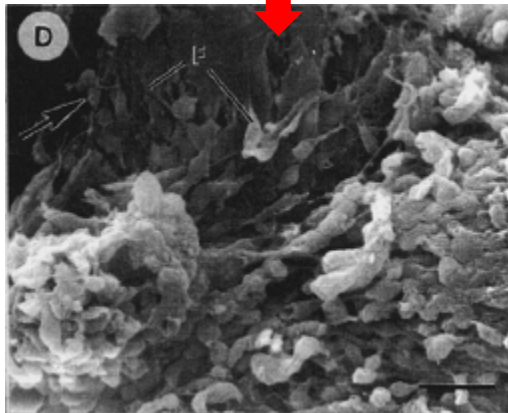
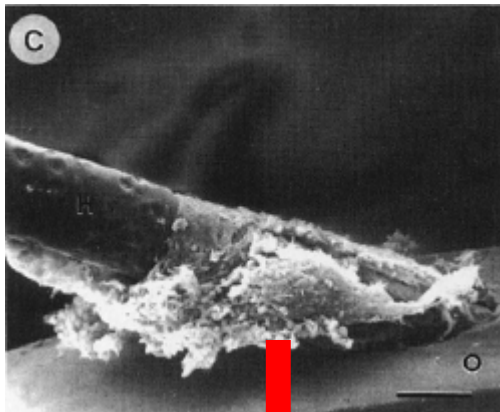
Susanne Haussler and Clay Fuqua. *Biofilms 2012: New Discoveries and Significant Wrinkles in a Dynamic Field*. J. Bacteriology Images courtesy of Annette Moter.

# Electron Microscopy



Direct observation of microbial colonization at high resolution using TEM or SEM:

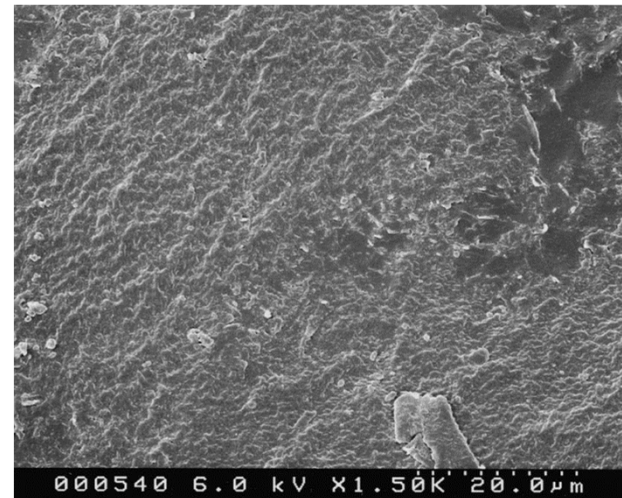
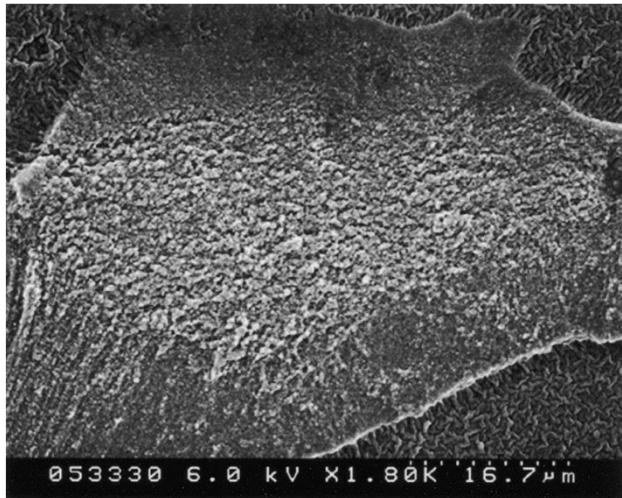
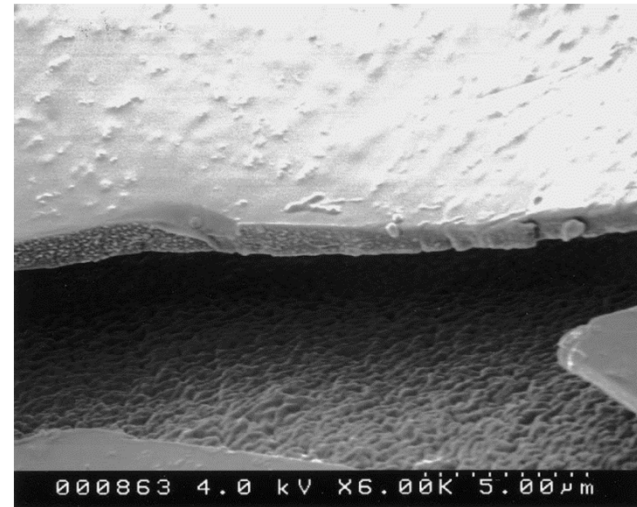
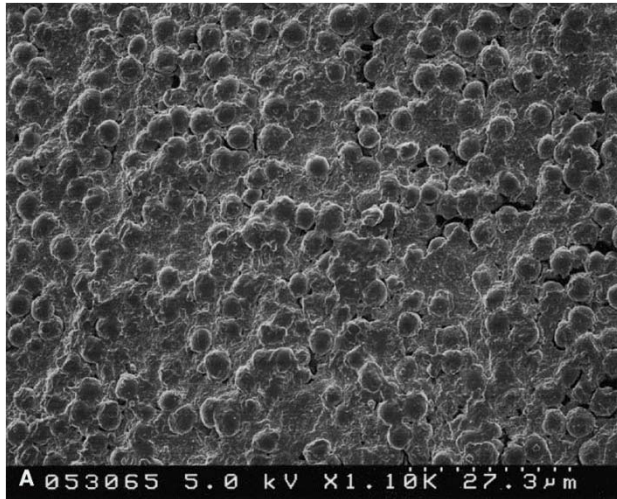
- Identification based on morphology or staining
- Additional methods required to confirm bacterial species



**SEM showing colonization of an intraocular lens from noninfected eyes Tanner, V. et al. (1998) J Cataract Refract Surg 24: 1145-51**

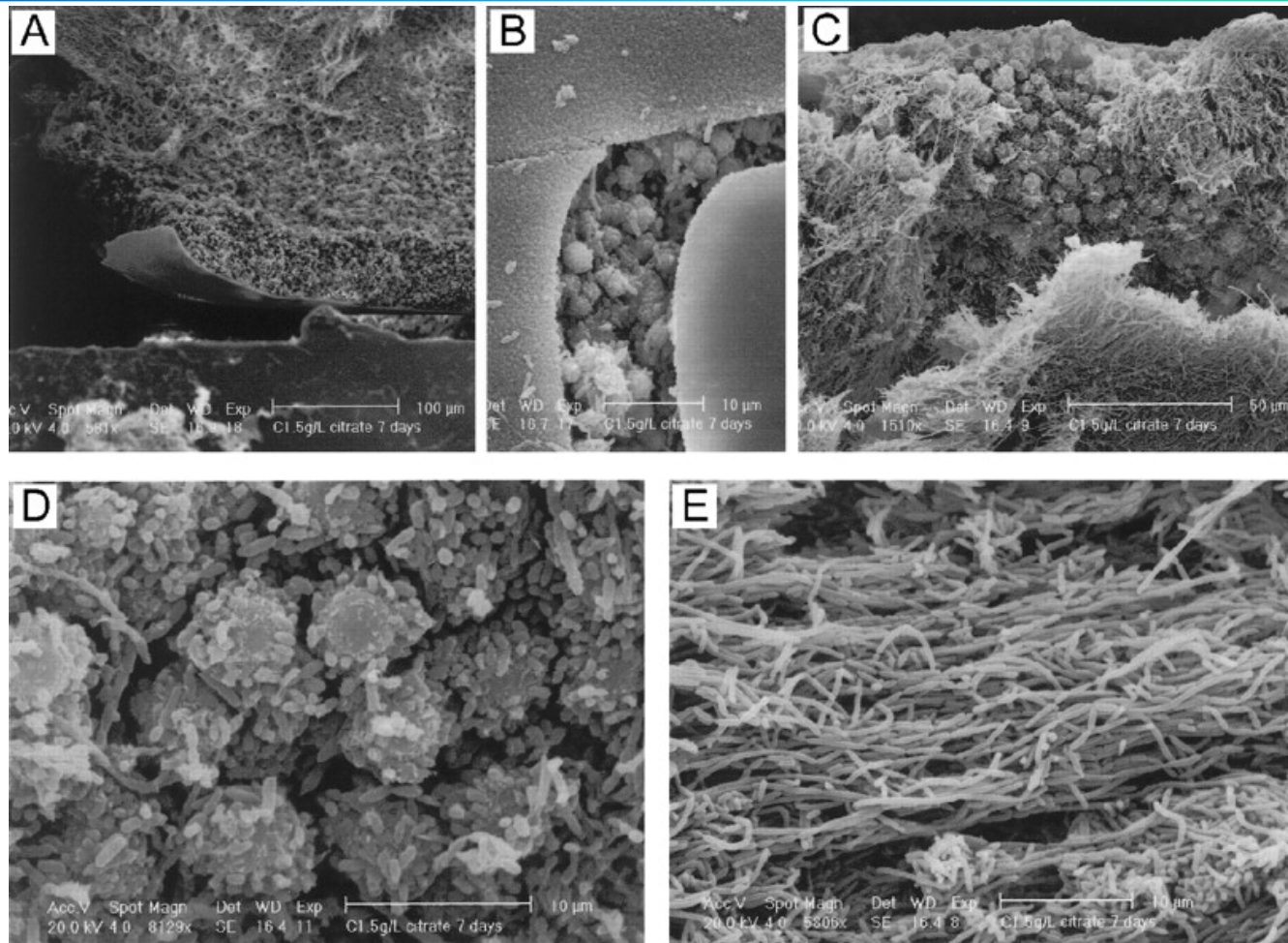


# Biofilms on infected cochlear devices



SEM showing cocci embedded in biofilm EPS matrix, and slimes of biofilm covering infected cochlear devices. Antonelli, P. et al. (2004) *Otology & Neurotology* 25:953-957

# *Proteus mirabilis* biofilm development on urinary catheters



David J. Stickler and Sheridan D. Morgan. 2006 Modulation of crystalline *Proteus mirabilis* biofilm development on urinary catheters. J Med. Microbiol.

# Needs in Biofilm imaging

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- High resolution
- Non-invasive
- Real-time
- Large area scanning
- Biofilms in natural environment (i.e. implants, mats)
- Functional imaging
- ...

# Other potential imaging tools

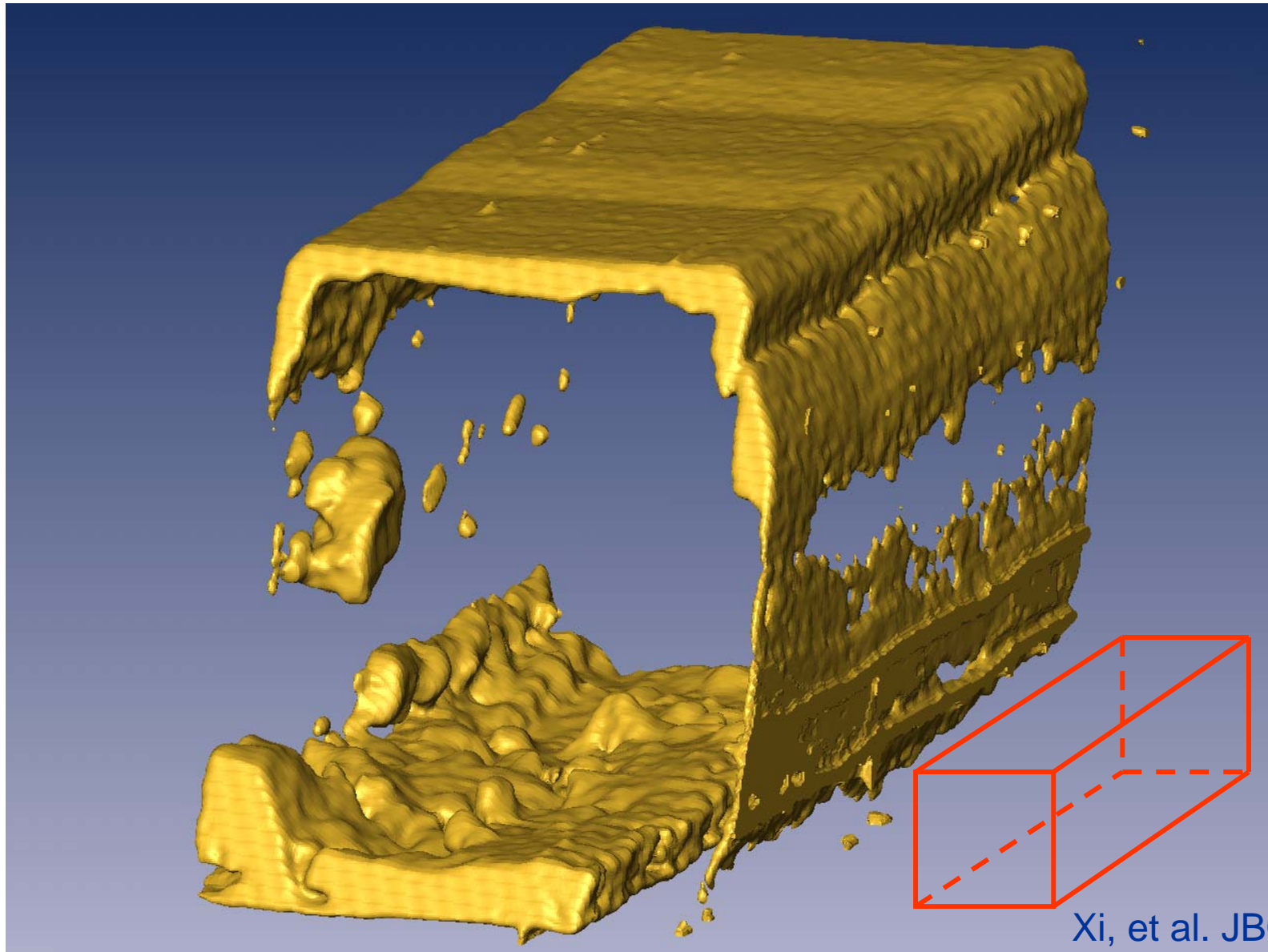
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- Magnetic resonance imaging (MRI)
- Optical coherence tomography (OCT)
- ...



# Optical Coherence Tomography (OCT)

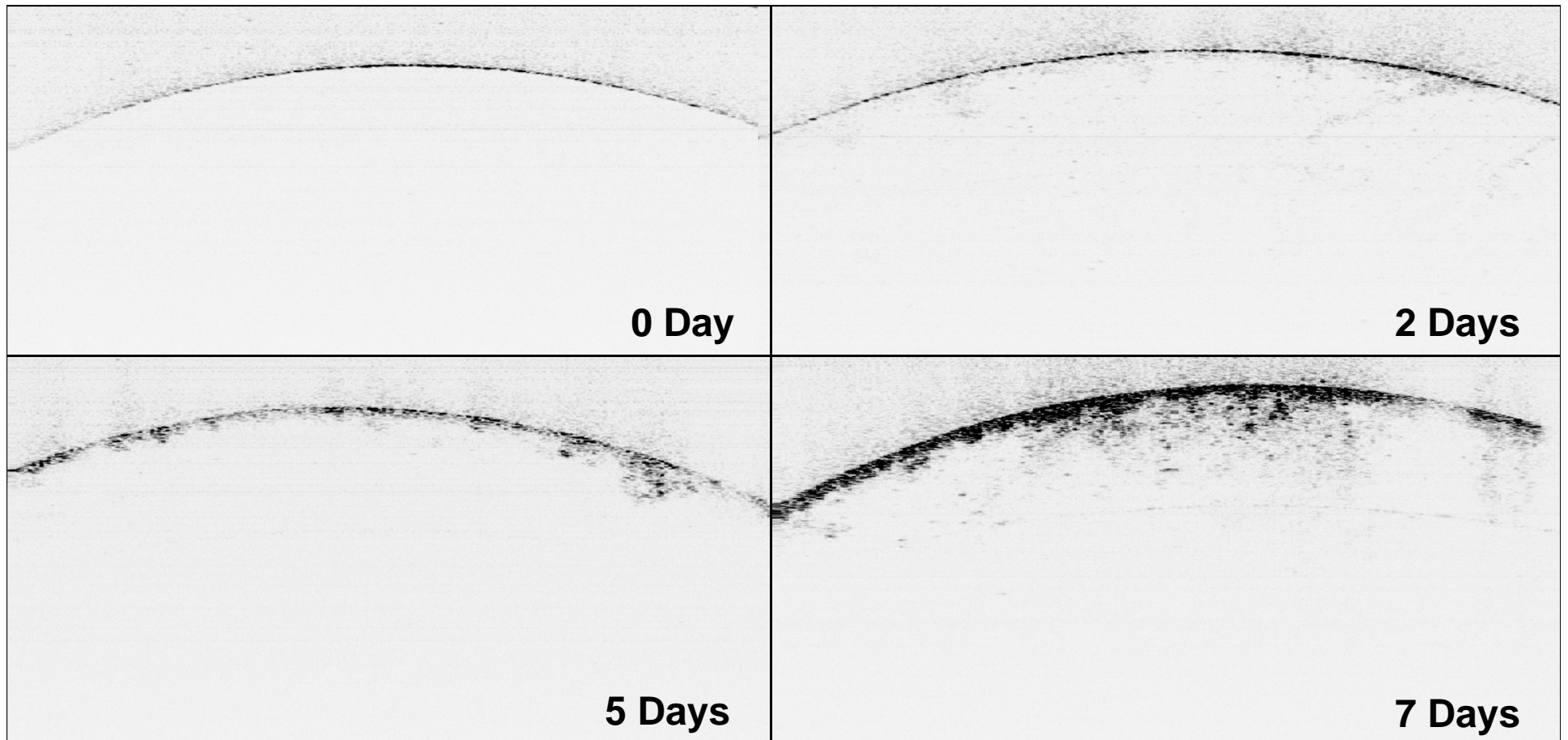
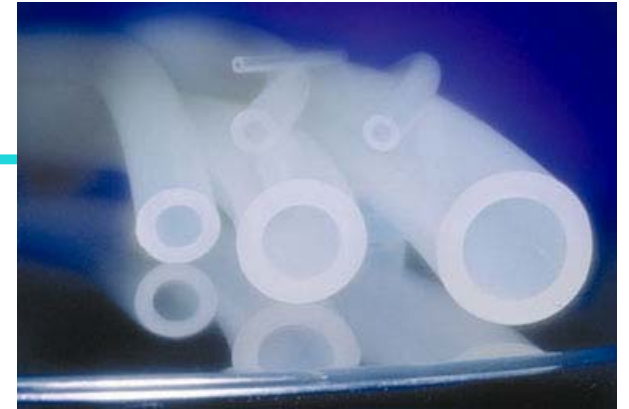


Xi, et al. JBO 2006

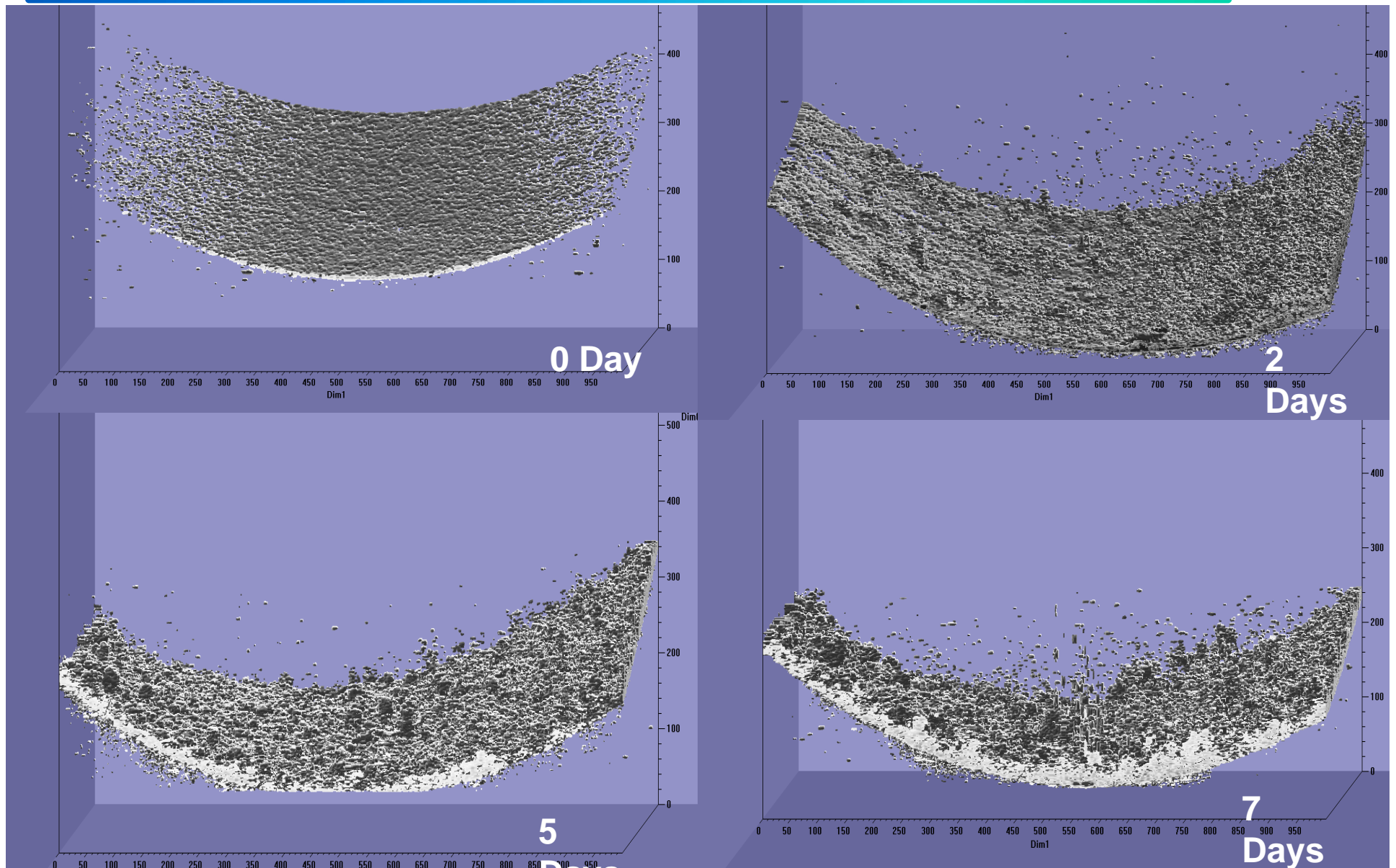


# ***In situ Biofilm imaging – OCT***

**Biofilms developed on the inner surface of a silicon tubing**



# *In situ Biofilm imaging – OCT*



# Future needs

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**Analysis of biofilms (structure, function, and molecular mechanisms) on implants *in vivo* (Joo and Otto, 2012)**

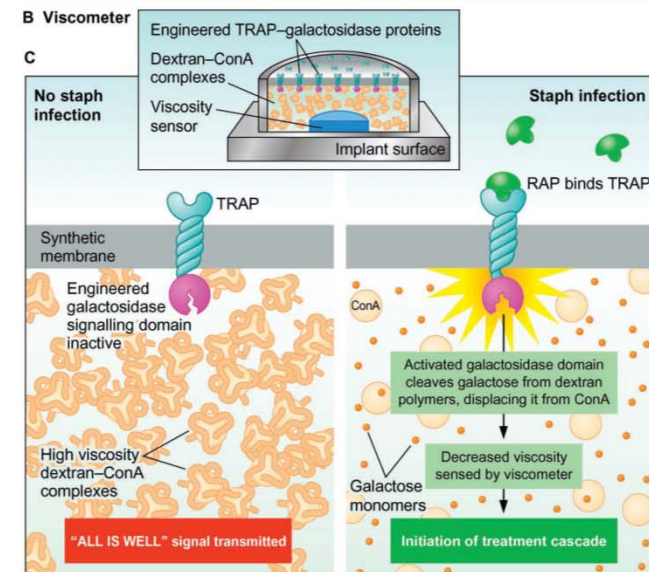
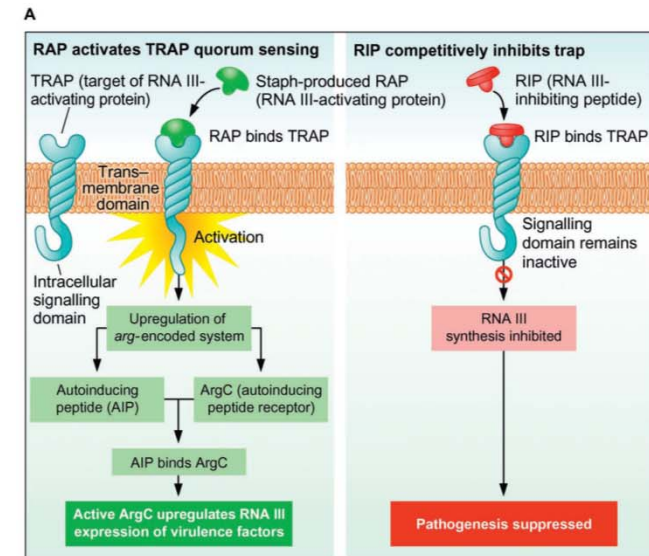
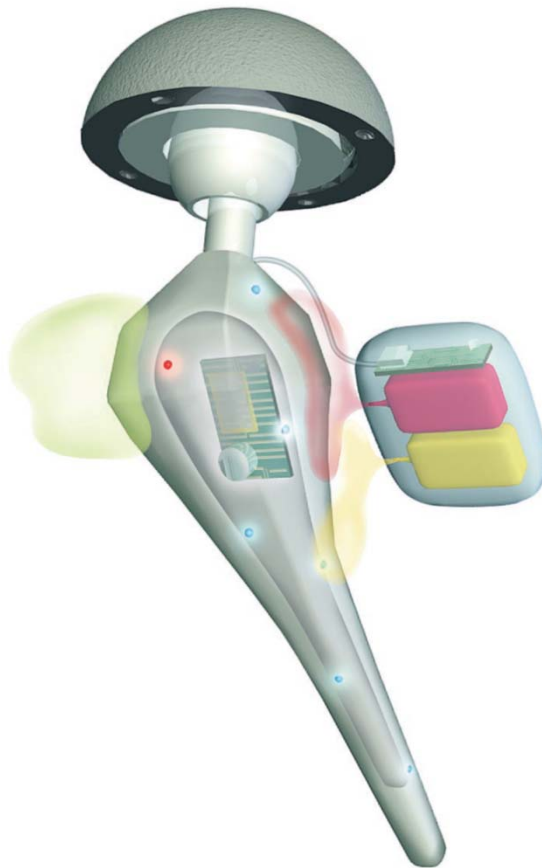
**Intelligent/smart devices for real time monitoring biofilms *in vivo***

**Biomarkers of biofilms**

**...**

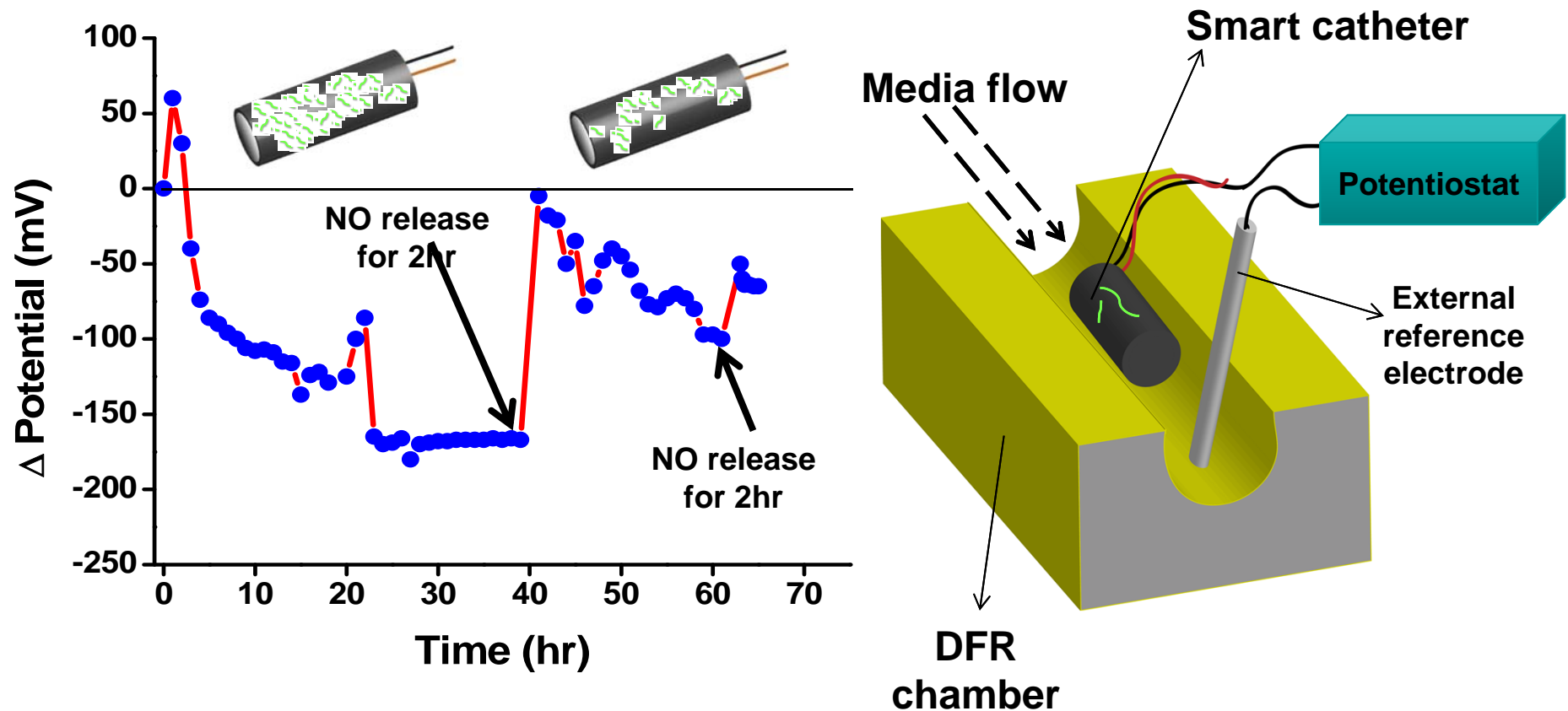


# Intelligent devices for real time monitoring Biofilms in vivo



Ehrlich, et al. 2004 Intelligent implants to battle biofilms.  
ASM news

# Smart Catheter



Meyerhoff, Xi, et al. Unpublished data

# Implications for anti-biofilm device development

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- Most of biofilms on medical devices are polymicrobial, tests on one or two model species may not be sufficient
- Need to take into consideration of biofilm matrix
- Host derived materials can be part of biofilm structures
- Effectiveness in a test model system *in vitro* may not be readily translated into clinical benefits
- ...